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## Flavonoid glycosides from *Periploca laevigata* (Asclepiadaceae) from Algeria

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### ABSTRACT

The phytochemical investigation of the *n*-butanol extract of aerial parts of *Periploca laevigata* resulted in the isolation of four known compounds, kaempferol 3-*O*- $\beta$ -arabinopyranoside (1), kaempferol 3-*O*- $\beta$ -glucopyranoside (2), quercetin 3-*O*-arabinopyranoside (3) and quercetin 3-*O*-rutinoside (4). The structures of the compounds were determined on the basis of extensive spectroscopic analysis, including 1D and 2D NMR as well as acid hydrolysis and comparison with the related known compounds.

**Key words:** flavonoid glycosides, *Periploca laevigata*, Asclepiadaceae.

### INTRODUCTION

Asclepiadaceae is a large family consisting about 175 - 180 genera and 22 000 species. The genus *Periploca* includes many species, from which *Periploca laevigata* Ait. This species is native to the Mediterranean region and widely distributed in the Sahara area [1]. In Algeria, it is predominantly found in the south of the country, especially in the Bechar region and Hogar [2] and locally known as Elhalab latex. *Periploca laevigata* is reputed to possess medicinal properties. In Algerian Sahara, it is used for the treatment of boils and buttons; whereas in Tunisia, it is taken as tea and used as herbal medicine for the treatment of headaches and diabetes [3]. Previous Tunisian phytochemical studies of this species, led to the isolation and identification of  $\alpha$ - and  $\beta$ -amyrin, lupeol,  $\beta$ -sitosterol and periplocadiol from the roots [4]. However the oleanolic acid, masilinic acid, 12 $\alpha$ -hydroxy- $\delta$ -lactone of oleanolic acid, arjunolic acid, Asiatic acid,  $\beta$ -D-glucopyranose and  $\alpha$ -D-glucopyranose have been isolated from fruit barks of *P. laevigata* [5-6]. Additional Tunisian phytochemical study led to the isolation and identification of lupeol arachidate, procrims A and B and laevigatins I and II together with lupeol and lupeol acetate [7].

In continuation of our study on Algerian medicinal species [8-11], we report here the isolation and the structure elucidation of flavonoid glycosides from the *n*-butanol fraction of the soluble part of the aqueous MeOH extract of the leaves of *Periploca laevigata* from Algerian Sahara. The structures of the isolated compounds were identified on the basis of spectroscopic studies, acid hydrolysis in comparison with literature data. Up to our knowledge, all these compounds are described for the first time from this species.

### MATERIALS AND METHODS

#### *Plant material*

*Periploca laevigata* was collected during the flowering phase in April 2005, in the south east of Algeria, and was authenticated by Mr. Benabdelhakem (Bechar Algeria) on the basis of Ozenda [2]. A voucher specimen has been

deposited in the Herbarium of the Unit Research VARENBIOMOL of frères Mentouri University of Constantine (PLA04/05).

#### Extraction Procedure

Dried and powdered aerial parts (210 g) of *Periploca laevigata* were extracted with a mixture of EtOH: H<sub>2</sub>O (7:3) three times for 24h. The EtOH extract was concentrated on a rotary evaporator under reduced pressure. This residue was dissolved in water (300 ml). After remove up to chlorophyll with petroleum ether (0.5 g), the remaining aqueous solution was extracted successively with CHCl<sub>3</sub>, EtOAc and *n*-BuOH. The obtained extracts were according to chloroform (4.8 g), ethyl acetate (2.9 g) and *n*-BuOH (10.5 g) respectively.

The *n*-BuOH extract was subjected to column chromatography on silica gel (70-230 mesh), eluted with CHCl<sub>3</sub>/MeOH in gradient polarity, yielding to 44 fractions (F1-F44) obtained by combining the eluates on the basis of TLC analysis. The fraction 16 (50.4 mg) was chromatographed over silica gel preparation plates and eluted with CH<sub>2</sub>Cl<sub>2</sub>: MeOH (8:1) to give **1** (19 mg). Fraction F26 (85 mg) was submitted to preparative TLC on silica gel GF254 eluting with CH<sub>2</sub>Cl<sub>2</sub>: MeOH (5:1) to offer **2** (35.2 mg). Moreover Fraction F27 (118 mg) was also subjected to preparative TLC on silica gel GF254 using the same eluate as fraction F26 to give 4 sub-fractions (F27-1 to F27-4). The sub-fraction F27-2 was purified over Sephadex column eluting with MeOH to give **3** (11 mg). Finally, the fraction F36 (954 mg) was chromatographed over preparative TLC on silica gel GF254 using EtOAc: MeOH: H<sub>2</sub>O (6:1:1) as eluent to give **4** (9.2 mg). The structures were elucidated using modern methods of analysis in particular UV spectrophotometer, NMR and its various experiments as well as acid hydrolysis. All these data were in good agreement with the respective literature data.

#### Acid hydrolysis

Solutions of compounds **2**, **3** and **4** in 2 ml (HCl 4N) were heated separately for 2 h and left to cool. Each mixture was extracted with EtOAc and the EtOAc fraction was used for detection of the aglycone. The aqueous fraction was concentrated and used for identification of sugars. The sugars were identified by TLC using solvent system (acetone–water; 90:10) by comparing with authentic samples.

### RESULTS AND DISCUSSION

From the *n*-butanol extract of *Periploca laevigata* aerial parts, four flavonoid glycosides (**1-4**), were isolated by chromatographic methods then identified on the basis of their UV and NMR spectral data and comparison with literature data for similar structures.

**Compound 1:** yellow amorphous powder soluble in methanol. UV  $\lambda_{\max}$  (nm): MeOH: 266, 350; + NaOH: 274, 327, 399; +AlCl<sub>3</sub>: 273, 305, 397; + AlCl<sub>3</sub>/HCl: 275, 302, 397; +NaOAc: 274, 304, 372; + NaOAc/H<sub>3</sub>BO<sub>3</sub>: 268, 306, 354. The <sup>1</sup>H NMR spectrum (CD<sub>3</sub>OD-d<sub>4</sub>, 400MHz,  $\delta$ :ppm, *J*:Hz) exhibited: 8.03 (2H, *d*, *J*=8.9, H-2'&H-6'), 6.85 (2H, *d*, *J*=8.9, H-3'&H-5'), 6.34 (1H, *d*, *J*=2, H-8), 6.14 (1H, *d*, *J*=2, H-6), 5.10 (1H, *d*, *J*=6.5, H-1'' anomeric proton of sugar), 3.86 (1H, *dd*, *J*=8.4; 6.5, H-2''), 3.60 (1H, *dd*, *J*=8.4; 3.3, H-3''), 3.75 (1H, *m*, H-4''), 3.45 (1H, *dd*, *J*=13.8; 3.5, H-5''a) and 3.72 (1H, *brd*, *J*=3.8, H-5''b) all these signals were deduced from COSY spectrum. The coupling constant H<sub>2</sub>''-H<sub>3</sub>'' (*J*=8.4Hz) indicated a diaxial configuration, whereas the coupling constant H<sub>3</sub>''-H<sub>4</sub>'' (*J*=3.3Hz) indicated an axial-equatorial orientation then an arabinopyranosyle configuration [12]. <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 100MHz,  $\delta$ :ppm): 179.8 (C-4), 164 (C-7), 161.3 (C-5), 160.5 (C-2), 134 (C-3), 156.9 (C-9), 158 (C-4'), 122.7 (C-1') deduced from HMBC spectrum), 132.2 (C-2' & C-6') and 116.3 (C-3' & C-5') deduced from HSQC spectrum), 105.0 (C-10), deduced from HMBC spectrum), 101.1 (C-6), 95.6 (C-8), 104.6 (C-1''), 72.7 (C-2''), 74.1 (C-3''), 69.0 (C-4''), 66.8 (C-5'') deduced from the HSQC spectrum. The pyranose skeleton is confirmed by the chemical shift of C-4'' at  $\delta$  69.0 ppm against 85.0 ppm for the furanose skeleton [12]. On the basis of all these results, this compound was identified as Kaempferol 3-*O*- $\beta$ -arabinopyranoside [13-14].

**Compound 2:** yellow powder soluble in methanol, UV  $\lambda_{\max}$  (nm): MeOH: 264, 365; + NaOH: 271, 330, 404; +AlCl<sub>3</sub>: 274, 438; + AlCl<sub>3</sub>/HCl: 268, 299, 360, 404; +NaOAc: 270, 373; + NaOAc/H<sub>3</sub>BO<sub>3</sub>: 263, 380. <sup>1</sup>H NMR (CD<sub>3</sub>OD-d<sub>4</sub>, 500MHz,  $\delta$ :ppm, *J*:Hz): 7.55 (1H, *dd*, *J* = 8.5; 2.1, H-6'), 7.71 (1H, *d*, *J* = 2.1, H-2'), 6.84 (1H, *d*, *J* = 8.5, H-5'), 6.36 (1H, *d*, *J* = 2.1, H-8), 6.16 (1H, *d*, *J*=2.1, H-6), 5.12 (1H, *d*, *J*= 6.8, H-1''anomeric proton of sugar). Acid hydrolysis of compound 2 produced quercetin and glucose. The *J*<sub>H-H</sub> coupling constant indicated that compound possesses  $\beta$ -linked glucose. This compound was characterized as quercetin-3-*O*- $\beta$ -glucopyranoside [15].

**Compound 3:** yellow amorphous powder soluble in methanol. UV  $\lambda_{\max}$  (nm): MeOH: 257, 357; + NaOH: 270, 329, 402; +AlCl<sub>3</sub>: 269, 433; + AlCl<sub>3</sub>/HCl: 264, 401; +NaOAc: 269, 389; +NaOAc/H<sub>3</sub>BO<sub>3</sub>: 264, 376. <sup>1</sup>H NMR (CD<sub>3</sub>OD-d<sub>4</sub>, 500MHz,  $\delta$ :ppm, *J*:Hz): 7.58 (1H, *dd*, *J* = 8.5; 2.1, H-6'), 7.74 (1H, *d*, *J* = 2.1, H-2'), 6.86 (1H, *d*, *J* = 8.5, H-5'), 6.37 (1H, *d*, *J* = 2, H-8), 6.18 (1H, *d*, *J*=2, H-6), 5.13 (1H, *d*, *J*= 6.8, H-1''anomeric proton of sugar), 3.10- 3.70

(sugar protons). Acid hydrolysis of compound 4 produced quercetin and arabinose. The configuration of anomeric sugar was deduced by its  $J_{H-H}$  coupling constant. This compound was characterized as quercetin 3-*O*-arabinopyranoside [16].

**Compound 4:** yellow amorphous powder soluble in DMSO. UV  $\lambda_{max}$  (nm): MeOH: 264, 364; + NaOH: 272, 330, 410; +AlCl<sub>3</sub>: 269, 277, 353, 374; + AlCl<sub>3</sub>/HCl: 263, 278, 354, 382; +NaOAc: 270, 434; +NaOAc/H<sub>3</sub>BO<sub>3</sub>: 269, 399. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 500MHz,  $\delta$ :ppm,  $J$ :Hz): 7.55 (1H, *dd*,  $J = 8.5$ ; 2.5, H-6'), 7.45 (1H, *d*,  $J = 2.5$ , H-2'), 6.74 (1H, *d*,  $J = 8.5$ , H-5'), 6.29 (1H, *brs*, H-8), 6.08 (1H, *brs*, H-6), which confirmed the identification of flavonol aglycone as quercetin. Two anomeric proton signals at 5.23 (1H, *d*,  $J = 7.0$ Hz) and 4.27 (1H, *brs*) were assignable to H-1 of a  $\beta$ -glucosyl proton and to the H-1 of an  $\alpha$ -rhamnosyl proton, respectively. A methyl signal 0.88 (3H, *d*,  $J = 6.5$ Hz) in the high-field region was assigned to CH<sub>3</sub> of rhamnose. Acid hydrolysis of compound 4 confirmed this hypothesis and produced quercetin, glucose and rhamnose. Consequently, this compound was characterized as quercetin 3-*O*- $\beta$ -rutinoside named rutin [17].

## CONCLUSION

The present study relates to the phytochemical investigation of the *n*-butanol extract of the aqueous-ethanolic extract obtained from the leaves of *Periploca laevigata*. This contribution led to the isolation of four known flavonoid glycosides named as *kaempferol 3-O- $\beta$ -arabinopyranoside*, *quercetin 3-O- $\beta$ -glucopyranoside*, *quercetin 3-O- $\beta$ -arabinopyranoside* and *quercetin 3-O-rutinoside*. All compounds are reported for the first time from this plant.

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